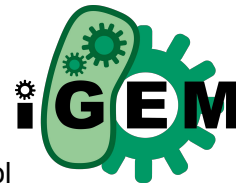




**UNSW**  
AUSTRALIA

BABS UNSW iGEM Lab Protocol



|              |  |                        |  |      |         |         |   |
|--------------|--|------------------------|--|------|---------|---------|---|
| Procedure    | Name   |                        | LacZ Assay   |      |         |         |   |
|              | Description  |                        | A LacZ assay for the expression of beta galactosidase  |      |         |         |   |
| Document     | Name   | Isabelle Capell-Hattam |  | Date | 18/0915 | Version | 1 |
| Requirements | Time   |                        | 4+ hours   |      |         |         |   |
|              | PPE  |                        | Gloves, Labcoat  |      |         |         |   |
|              | Equipment  |                        | Pipettes<br>Centrifuge<br>Spectrophotometer (420 nm)<br>Heat block   |      |         |         |   |
|              | Materials  |                        | Bacterial cultures (with measured OD)<br>1M Na2CO3<br><b>Permeabilization Solution</b><br>100 mM dibasic sodium phosphate (Na2HPO4)<br>20 mM KCl<br>2 mM MgSO4<br>0.8 mg/mL CTAB (hexadecyltrimethylammonium bromide)<br>0.4 mg/mL sodium deoxycholate<br>5.4 µL/mL beta-mercaptoethanol<br><b>Substrate Solution</b><br>60 mM Na2HPO4<br>40 mM NaH2PO4<br>1 mg/mL o-nitrophenyl-β-D-Galactoside (ONPG)<br>2.7 µL/mL β-mercaptoethanol |      |         |         |   |
| Step 1       | Pellet 1ml of culture and remove supernatant                 |                        |  |      |         |         |   |
| Step 2       | Resuspend supernatant in 100 µl of Permeabilization solution |                        |  |      |         |         |   |
| Step 3       | Add 600 µl of substrate solution and incubate at 37°C        |                        |  |      |         |         |   |

|                 |  |
|-----------------|--|
| Step 4          | Once a colour change has occurred pellet assay and add 700 µl of 1M Na <sub>2</sub> CO <sub>3</sub>  |
| Step 5          | Measure the absorbance at 420 nm   |
| Step 6          | Normalize absorbance to OD   |
| Notes           | This protocol is adapted from<br><a href="http://openwetware.org/wiki/Beta-Galactosidase_Assay_(A_better_Miller)">http://openwetware.org/wiki/Beta-Galactosidase_Assay_(A_better_Miller)</a> |
| Version History |  |